

(0.1% trifluoroacetic acid) in 30 minutes. Peptide identity was confirmed by Electrospray Mass Spectrometry (Perkin-Elmer-Sciex API-3). Quantitative amino acid analysis was performed to show the correct ratio of amino acids and to determine the peptide content for calculation of the final concentration.

EXAMPLE 4

10 Preparation of Solutions

LTB₄ was dissolved in ethanol and diluted with HBSS (pH 7.3) to a final ethanol concentration of 0.001%. Synthetic complementary peptides and synthetic chemoattractants were dissolved in HBSS (pH 7.3). When necessary, the osmolality was adjusted between 280 and 320 mOsm by adding a small amount of distilled water.

EXAMPLE 5

Ultrafiltrate from Alkali-Degraded Rabbit Corneas

5 Ultrafiltered tripeptide chemoattractants were obtained from alkali-degraded rabbit corneas. Corneal buttons were excised from rabbit eyes (Pel-Freez Biologicals, Rogers, AR) using an 11 mm trephine. Based on an average dry weight of 11 mg/cornea in a preliminary experiment, corneas were placed in a known amount of

10 1.0 N NaOH (83.34 mg corneal dry weight/ml alkali, 1:12) for 24 hours at 37°C. The resultant suspension was titrated to pH 7.4 with

1.0 N HCl. This yielded a crude extract containing 41.67 mg corneal dry weight/ml of neutralized alkali. Briefly, the purification technique involved ultrafiltration (30,000, 3,000, and 1,000

15 Molecular Weight cutoff membranes in sequence) and dialysis (100 MWt cutoff membrane) of this crude extract.⁴ The final ultrafiltrate was lyophilized and the powder dissolved in HBSS to a final concentration of 83.34 mg corneal dry weight/ml. This concentration was based on the original mg of corneal dry weight exposed to alkali.

According to a previous study⁴, the ultrafiltered chemoattractant sample was composed of small peptides between 100 and 1,000 MWt. The only chemoattractants in this ultrafiltrate were N-acetyl-PGP and N-methyl-PGP. The specific activity of N-
5 acetyl-PGP was found to be superior.

EXAMPLE 6

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Neutrophil Isolation

These experiments followed the tenets of the Declaration of Helsinki and were approved by the human research committee at Brookwood Medical Center. All donors signed written consent forms
15 that explained the nature and possible consequences of the study. Blood was collected from only one donor each day.

Following the technique of Ferrante and Thong¹³, polymorphonuclear leukocytes were isolated from fresh heparinized human whole blood by centrifugation on Hypaque-Ficoll (density =